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**STATE-OF-THE-ART PAPER**

# Hormone Replacement Therapy and the Cardiovascular System

## Lessons Learned and Unanswered Questions

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Cardiovascular disease is the leading cause of death among women in the U.S., exceeding breast cancer mortality in women of all ages. Women present with cardiovascular disease a decade after men, and this has been attributed to the protective effect of female ovarian sex hormones that is lost after menopause. Animal and observational studies have shown beneficial effects of hormone therapy when it is initiated early in the perimenopausal period or before the development of significant atherosclerosis. However, randomized, placebo-controlled trials in older women have not shown any benefit in either primary prevention or secondary prevention of cardiovascular events, with a concerning trend toward harm. This review outlines the lessons learned from the basic science, animal, observational, and randomized trials, and then summarizes yet-unanswered questions of hormone therapy and cardiovascular risk. (J Am Coll Cardiol 2006;47:1741–53) © 2006 by the American College of Cardiology Foundation

Cardiovascular disease is the leading cause of death among women in the U.S., accounting for more than 500,000 deaths annually (1). Mortality attributable to coronary heart disease (CHD) exceeds breast cancer mortality in women at all ages (2). The vast majority of these cardiovascular events occur in postmenopausal women. Heart disease develops in women on average 10 years later in life compared with men, and this lag has been attributed to the protective effects of female sex hormones, particularly estrogens, before menopause (3).

Initially, data from animal studies and observational studies such as the Nurses' Health Study (4) strongly supported a protective cardiovascular benefit of hormone therapy (HT) after menopause, an effect not supported by randomized placebo-controlled trials in both secondary prevention (5) and primary prevention (6), which instead showed a concerning trend toward harm. However, many unanswered questions remain.

This review outlines lessons learned from basic science of estrogen action and animal studies and from observational and randomized trials, followed by a discussion of as-yet-unanswered questions about HT and cardiovascular risk.

## MOLECULAR AND CELLULAR BASIS OF ESTROGEN IN VASCULAR BIOLOGY

Estrogen can have both positive and negative effects on the cardiovascular system (7) (Fig. 1). On the positive side, estrogen has potentially beneficial effects on lipid parameters, such as reducing low-density lipoprotein cholesterol (LDLC) and increasing high-density lipoprotein cholesterol (HDLC), facilitating nitric oxide-mediated vasodilation, and inhibiting the response of blood vessels to injury and the development of atherosclerosis (8). On the negative side, estrogens increase triglycerides (9,10) and inflammatory markers such as C-reactive protein (CRP) (11,12). Estrogen also has many prothrombotic effects, such as increasing circulating levels of prothrombin and decreasing antithrombin III (13,14), contributing to an increased risk of venous thromboembolic events. Importantly, many of these effects of estrogen are mediated by first-pass effects on the liver, and thus result from oral but not transdermal administration of HT. For example, increased levels of CRP seem to occur only with oral estrogen administration. The extent to which this is associated with an increase in cardiovascular disease risk is uncertain (15,16). These observations underscore the potential importance of the mode of administration on the overall effects of HT on CHD risk as discussed below.

## MECHANISMS OF ESTROGEN ACTION

As our understanding of the mechanisms by which estrogens affect the cardiovascular system has increased, it has become clear that the complexity of the biological effects of estrogens are reflective of quite complex mechanisms of action (7,8). As noted above, estrogens regulate a variety of systemic or circulating factors, including lipids, inflammatory factors, and members of the coagulation/fibrinolytic cascades. Estrogens can also act directly on the heart and the vasculature. The effects of estrogen are mediated by estrogen receptors, of which two are known, ER$\alpha$ and ER$\beta$, and

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**Abbreviations and Acronyms**

| | |
|---|---|
| CHD | = coronary heart disease |
| CI | = confidence interval |
| CRP | = C-reactive protein |
| HDLC | = high-density lipoprotein cholesterol |
| HT | = hormone therapy |
| IMT | = intima-medial thickness |
| LDLC | = low-density lipoprotein cholesterol |
| MI | = myocardial infarction |
| MPA | = medroxyprogesterone acetate |

both are expressed in cardiovascular cells and tissues (7,8). Estrogen receptors are classically thought of as ligand-activated transcription factors that reside in the cell nucleus and regulate gene expression in response to hormone binding. This mechanism, often referred to as the genomic pathway, likely underlies the longer-term effects of estrogen, such as those on circulating levels of lipids and coagulation factors. More recently it has become clear that estrogen receptors also transduce the rapid effects of estrogen that occur within minutes (17) and are referred to as non-genomic because they do not depend on changes in gene expression. These rapid effects of estrogen are mediated by a subpopulation of estrogen receptors localized to cell membrane signaling domains called caveolae. The best-studied example of this non-genomic pathway for estrogen action in the cardiovascular system is the activation of endothelial cell nitric oxide synthase that results in arterial vasodilation in response to acute administration of estrogen.

## IMPACT OF DISEASE STATE ON THE CARDIOVASCULAR EFFECTS OF ESTROGEN

There is growing evidence showing that the effects of estrogen on the vasculature depend in part on the extent to which atherosclerosis has become established. For example, estrogen receptor expression is markedly diminished in atherosclerotic arteries (18,19), and thus, to the extent that direct, receptor-dependent effects on the vasculature contribute to the potential for anti-atherosclerotic effects, this will be diminished or absent in diseased arteries. In addition, the effects of estrogen on a given pathway may have different consequences depending on the state of health of

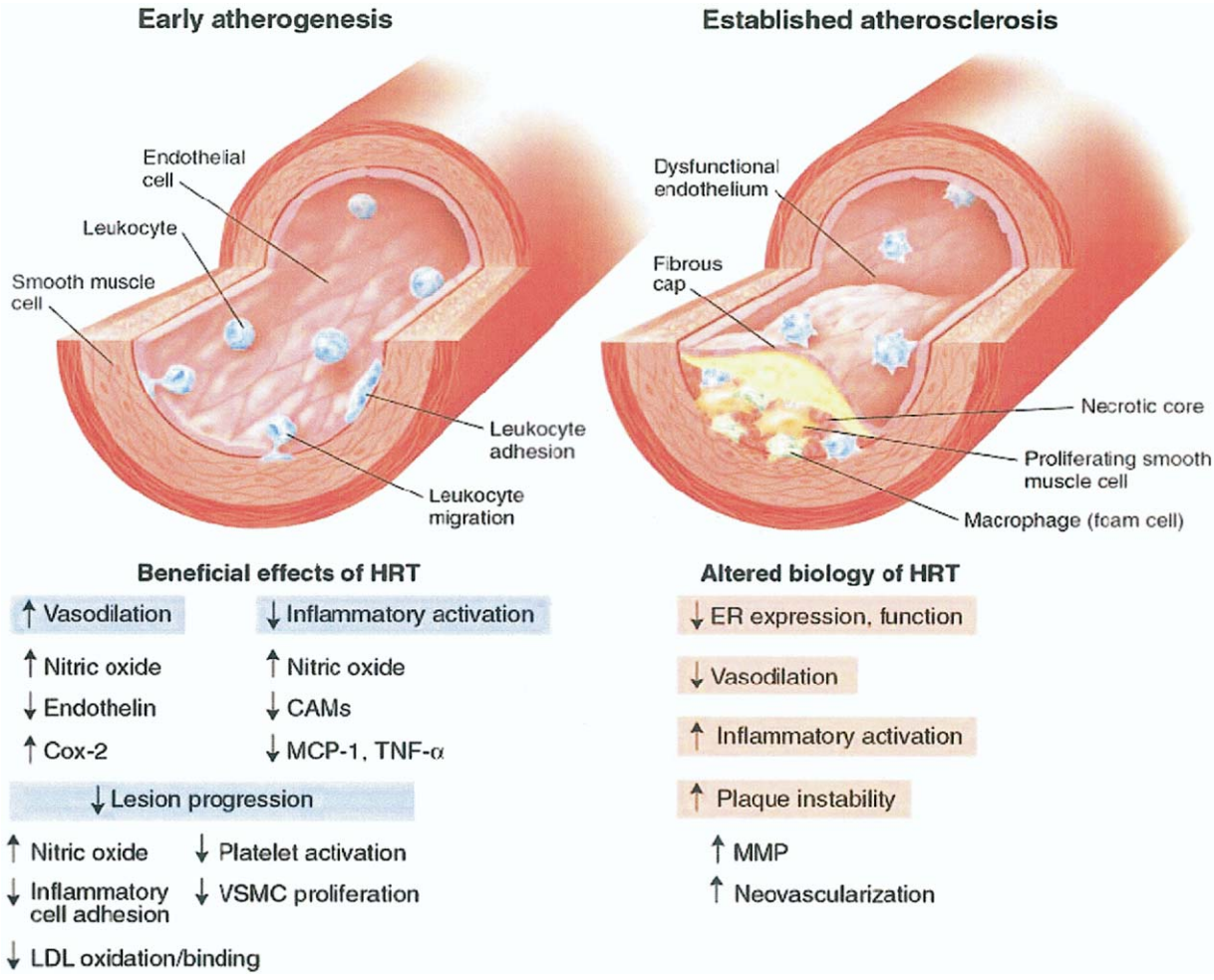


**Figure 1.** Estrogen: beneficial and thrombogenic effects. CAM = cell adhesion molecule; Cox-2 = cyclooxygenase 2; ER = estrogen receptor; HRT = hormone replacement therapy; LDL = low-density lipoprotein; MCP = monocyte chemoattractant protein; MMP = matrix metalloproteinase; TNF = tumor necrosis factor; VSMC = vascular smooth muscle cell. Reprinted, with permission, from Mendelsohn and Karas (7).

the underlying vessel. For example, estrogen up-regulates specific members of the matrix metalloproteinase (MMP) family such as MMP-9 (20). The MMPs degrade the extracellular matrix with the arterial wall. Thus, in a non-diseased artery, an estrogen-induced increase in MMP-9 may have little or no consequences, whereas in an atherosclerotic artery, where MMP-9 is expressed in the shoulder region of an atherosclerotic plaque, an increase in MMP-9 activity could conceivably be associated with an increased risk of plaque rupture and thus acute coronary syndromes. The Fitzgerald laboratory has recently shown that estrogen-mediated up-regulation of cyclooxygenase-2 plays an important role in retarding atherosclerosis in a hypercholesterolemic mouse model (21). This suggests that atherosclerotic arteries with impaired cyclooxygenase-2 responses may also lose this potentially beneficial effect of hormone treatment.

More direct support for the hypothesis that the effects of estrogen on cardiovascular risk depend on the timing of initiation of therapy in relation to the extent of underlying atherosclerosis comes from the Clarkson laboratory. Using a well-established monkey model of atherosclerosis, Clarkson and colleagues have shown that the antiatherosclerotic effects of oral conjugated equine estrogens (CEE) are apparent only in monkeys with minimal underlying atherosclerosis at the time that therapy is initiated, as reviewed in Karas and Clarkson (20), a finding also supported by rodent and rabbit studies (22,23).

## CARDIOVASCULAR DISEASE AND MENOPAUSE

Interest in the role estrogen plays in cardiovascular disease was stimulated by the observed increase in cardiovascular events after menopause. In 1976 the Framingham investigators reported a 2.6-fold higher incidence of cardiovascular events in age-matched postmenopausal women compared with premenopausal women (24). The excess CHD risk associated with surgical menopause was 2.7-fold higher compared with premenopausal women of the same age ($p < 0.01$) (25) and 2.2-fold higher compared with women with a natural menopause. This excess risk seemed to be prevented by estrogen replacement therapy (26). Plasma lipoproteins were thought to play a role in the increased CHD risk that menopause confers because total cholesterol, LDL cholesterol, and triglyceride levels all increase in women after menopause (27), and HT seemed to counter these unfavorable effects of lipids, although the cardioprotective HDL cholesterol levels also decreased (28). In addition, there is an age-associated increase in the incidence of cardiovascular disease for both premenopausal and postmenopausal women independent of the effects of HT. Overall, however, data indicate that withdrawal of estrogen during menopause is associated with an increased risk of heart disease above that seen for premenopausal women. This led to interest in the potential cardiovascular benefit from postmenopausal estrogen replacement therapy.

## ANIMAL STUDIES OF HORMONE REPLACEMENT

In animal studies, estrogens exert vasodilator (29), anti-inflammatory (30), and antiatherosclerotic (31) properties, as well as favorably affecting lipid profiles. In a study of randomized ovariectomized hypercholesterolemic rabbits, estradiol significantly reduced atherosclerosis progression compared with levonorgestrel or no hormones (32). A series of studies have also shown that estradiol significantly lessens the response to vascular injury in mice and further implicate ER$\alpha$ as the specific estrogen receptor that mediates this vasculoprotective effect (33–36). Similarly in ovariectomized monkeys, 17$\beta$-estradiol or CEE reduced coronary artery atherosclerosis compared with control animals by 50% ($p \leq 0.05$) (37) to 72% ($p < 0.04$) (38). Although the role of estrogen replacement seemed promising in the animal studies, the data regarding progesterone were more conflicting (39).

## OBSERVATIONAL TRIALS OF HORMONE REPLACEMENT

Overall, the animal studies suggested a promising role of estrogen replacement after menopause. Simultaneously, a series of observational and case-control trials also suggested benefit (reviewed in Table 1). The majority of the smaller case-control studies (40–45) showed nonsignificant trends toward reduction in CHD events with overall odds ratios ranging from 0.69 to 0.9. However, a large case-control study did show a significant association with HT and reduced incidence of first myocardial infarction (MI) (46). Longer duration of use seemed to confer even more cardiovascular benefit (47).

In addition to reducing CHD events, cross-sectional data suggested less subclinical atherosclerosis in HT users (48). In an observational analysis of the Cardiovascular Health Study of women >65 years of age, estrogen users had lower levels of subclinical disease as measured by a variety of surrogate end points (49). Even more promising, the much larger observational Nurses' Health Study of 70,000 asymptomatic women showed a lower incidence of CHD events and all-cause mortality in HT users compared with nonusers (4,50,51). It is important to note that most women in the Nurses' Health Study likely started taking HT in the perimenopausal period and were free of known CHD at the start of the study.

## RANDOMIZED TRIALS OF HORMONE REPLACEMENT THERAPY

The animal and observational studies were followed by a series of randomized, placebo-controlled trials of HT in both primary and secondary prevention, and with both surrogate and cardiovascular event outcomes, which failed to confirm cardiovascular benefit (reviewed in Table 2). Although two smaller randomized trials using the surrogate end points of carotid intima-medial thickness (IMT) (52) and brachial reactivity (53) favored estrogen, three other

**Table 1.** Observational and Case-Controlled Studies of Hormone Therapy

| Investigator/ Year | Type of Study | Population | Hormone Preparation | Primary End Point | Results | Conclusions |
|---|---|---|---|---|---|---|
| Rosenberg et al. (40)/1993 | Case-controlled | 858 women age 45–60 yrs with first MI compared with 858 age-matched control subjects. | Estrogen (E) alone: 21% of both cases and control subjects used E; most using CEE. | First MI | OR of 0.9 (0.7–1.2) with history of E use. For >5 yrs of use OR of 0.6 ($p = 0.08$). | Nonsignificant trend toward reduced first MI in E users, longer-term use was stronger than recent use ($p < 0.05$) and compared with past use ($p = 0.08$). |
| Mann et al. (41)/1994 | Case-controlled | Database within British National Health Service. Women age 45–64 yrs (n = 567,096), with 1,521 cases of MI matched with 6,084 control subjects. | Any E or E+ progestin (P): 117 cases and 562 control subjects used HT. Approximately 2/3 on E+P and 1/3 on E. | First MI (fatal or nonfatal) | OR 0.83 (0.66–1.03), $p = 0.089$ with HT use. Nonsmokers on HT, OR 0.70 (0.49–1), smokers on HT, OR 1.05 (0.71–1.53). | Nonsignificant trend toward reduced first MI with any form of HT. However, protective effect seems to be confined to nonsmokers. |
| Psaty et al. (42)/1994 | Case-controlled | Group Health Cooperative of Puget Sound, WA. Postmenopausal women, 502 cases of MI and 1,193 control subjects. | Any E or E+P. Among cases, HT use was E (n = 45) and E+P (n = 16); among control subjects use was E (n = 157) and E+P (n = 74). Majority used cyclical 0.625 CEE and 10 mg MPA. | First MI (fatal or nonfatal) | OR 0.69 (0.47–1.02) with E alone. OR 0.68 (0.38–1.22) with E+P. | Nonsignificant trend toward reduced risk of MI. |
| Jonas et al. (48)/1996 | Cross-sectional, nonrandomized | 2,962 women in the Cardiovascular Health Study. | E formulation not specified. Past users (n = 787), current E alone (n = 280), current E+P (n = 73). | Carotid IMT Carotid stenosis | IMT was 0.22 mm less in E ($p = 0.003$) and 0.09 mm less in E+P ($p = 0.05$) vs. in nonusers. Adjusted OR for carotid stenosis of 0.61 (0.36–1.01) for E and OR 0.91 (0.67–1.25) for E+P. | Both E+P and E alone were associated with decreased measures of carotid atherosclerosis. |
| Grodstein et al. (4)/1996 | Prospective observational | 59,337 women from Nurses' Health Study ages 30–55 yrs at baseline. 770 cases of MI/ CHD death and 572 cases of stroke over 16-yr follow-up. | Past users (n = 12,503), current E alone (n = 7,776), and E+P (n = 6,224). | MI or CHD death | For MI/CHD events, RR 0.39 (0.19–0.78) in E+P, 0.60 (0.43–0.83) in E alone. For stroke, RR 1.09 (0.66–1.8) in E+P and 1.27 (0.95–1.69) for E alone. | These data support a reduced risk of hard CHD events in women on HT that is not attenuated by the addition of progestin. However, there was a nonsignificant trend toward increased strokes. |

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**Table 1.** Continued

| Investigator/ Year | Type of Study | Population | Hormone Preparation | Primary End Point | Results | Conclusions |
|---|---|---|---|---|---|---|
| Grodstein et al. (50)/1997 | Prospective observational | Postmenopausal women of Nurses' Health Study. 3,637 cases and 34,625 control subjects over 18-yr follow-up. | Any hormone replacement. | Mortality | RR 0.63 (0.56–0.70) in current HT users, decreasing after 10 years of use (RR 0.80, 0.67–0.96). Benefit seen in HT users with CHD risk factors (RR 0.51, 0.45–0.57), not in those at low risk (RR 0.89, 0.62–1.28). | Mortality is lower among current HT users; however, survival benefit diminishes over time and is lower for women at low risk for CHD. |
| Heckbert et al. (47)/1997 | Case-controlled | Group Health Cooperative of Puget Sound, WA enrollees. Postmenopausal women; 850 cases with MI and 1,974 control subjects. | E alone or E+P. 229 cases and 700 control subjects used HT, most commonly CEE with or without MPA. | Fatal or nonfatal MI | For categories of duration of E use, OR 1.0 for never (ref), 0.91 for <1.8 yrs, 0.70 for 1.8–4.2 yrs, 0.64 for 4.2–8.2 yrs, and 0.55 for >8.2 yrs. $p = 0.05$ for the trend. | A longer duration of HT among current users was associated with a reduced risk of first MI. |
| Sidney et al. (43)/1997 | Case-controlled | Kaiser database. Postmenopausal women age 45–74 yrs; 438 cases with MI and 438 age-matched control subjects. | E or E+P. In women s/p hysterectomy 51% used E, 1.2% used E+P. In women with a uterus 18.4% used E+P, 3% used E. | MI | OR 0.96 (0.66–1.49) in current HT users compared with nonusers, OR 1.07 (0.72–1.58) in past users. | No statistically significant decrease in OR for MI in current or past users of HT. |
| Petitti et al. (44)/2000 | Case-controlled | Same population of Kaiser database as above. | As above. | MI | OR 0.9 (0.5–1.6) in current HT users without CHD risk factors, 0.8 (0.5–1.8) with 1 risk factor and 1.1 (0.5–2.2) with 2 risk factors. | No decrease in risk of MI in current users of HT who had 0, 1, 2, or 3 major CHD risk factors. |
| Grodstein et al. (45)/1999 | Case-controlled | Sweden. Postmenopausal women with 213 cases of MI and 289 strokes matched to control subjects. | Medium-potency compared with low-potency or short-term E or E+P use. | MI and stroke | For MI, OR 0.75 (0.56–0.99) for medium-potency compared with low-potency E and OR 0.69 (0.45–0.90) for combined E+P. For stroke, OR 0.91 (0.71–1.17) for medium-potency E and 0.81 (0.61–1.10) for E+P. | Decreased risk of MI for medium potency E or E+P. No effect was seen on stroke risk. |

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**Table 1.** Continued

| Investigator/ Year | Type of Study | Population | Hormone Preparation | Primary End Point | Results | Conclusions |
|---|---|---|---|---|---|---|
| Grodstein et al. (51)/2000 | Prospective, observational | Nurses' Health Study. 70,533 postmenopausal women with 1,258 fatal/nonfatal MI and 767 strokes over 20-yr follow-up. | Any HT including CEE 0.3 mg, 0.625 mg, and >1.25 mg either alone or in combination with progestin. | Fatal or nonfatal MI and stroke | CHD events: RR in current E users of 0.61 (0.52–0.71); 0.54 (0.44–0.67) with CEE 0.625 mg and 0.58 (0.37–0.92) with CEE 0.3 mg. Stroke: RR 1.35 (1.08–1.68) with CEE 0.625 mg, 1.63 (1.18–2.26) for >1.25 mg, and 1.45 (1.10–1.92) for E+P. | CEE seemed to decrease the risk of CHD events with similar reduction for 0.3 mg and 0.625 mg CEE. However, CEE ≥0.625 mg or in combination with progestin may increase risk of stroke. |
| Varas-Lorenzo et al. (46)/ 2000 | Case-controlled | General Practice Research Database (n = 164,769), with 1,242 cases of first MI and 5,000 age-matched control subjects. | Any HT including oral (79%) and transdermal (21%) formulations. | MI | Current HT users had OR 0.72 (0.59–0.89), OR 0.52 (0.35–0.78) for E, and OR 0.79 (0.59–1.08) for E+P. | Data showed an association between HT and reduced incidence of acute MI. This was similar in users of oral and transdermal formulations. |
| Ferrara et al. (72)/2003 | Observational | Kaiser database. Diabetic women age >50 yrs (mean age 65 yrs, n = 25,000). | Low-, medium-, or high-dose E alone or combined E+P. There were 2,526 (10%) women on E alone and 2,088 (9%) on E+P. | 3-yr MI risk | In those without a recent MI, RH for MI for combined HT was 0.77 (0.61–0.97); unopposed estrogen was 0.88 (0.73–1.05). In those with a recent MI, RH was 1.78 (1.06–2.98). | In diabetic women without a recent MI, use of HT was associated with decreased risk of MI in women on <0.625 mg CEE but not a higher dose. However, HT was associated with an increased risk of MI in women with a history of recent MI, especially for HT use <1 yr. |

CEE = conjugated equine estrogens; CHD = coronary heart disease; E = estrogen; E+P = estrogen plus progestins; HR = hazard ratio; HT = hormone therapy; IMT = intimal-medial thickness; IV = intravenous; MI = myocardial infarction; MPA = medroxyprogesterone; OR = odds ratio (95% confidence interval); PE = pulmonary embolism; PO = per os (oral); RF = risk factors; RR = relative risk; TIA = transient ischemic attack.

**Table 2.** Summary of the Randomized Trials of Hormone Therapy

| Investigator/ Year | Study Design | Subjects | Intervention | Median Follow-Up | End Point | Results | Conclusions |
|---|---|---|---|---|---|---|---|
| Herrington et al. (54)/2000 (ERA) | Randomized, double-blind, placebo-controlled secondary prevention trial | 309 postmenopausal women with coronary stenosis >30%, mean age 65.8 yrs | CEE 0.625 mg only (n = 100), CEE 0.625+MPA 2.5 mg daily (n = 104), or placebo (n = 100) | 3.2 yrs | Quantitative coronary angiography: adjusted change in mean luminal diameter | −0.09 ± 0.02 mm for E, −0.12 ± 0.02 for combined E+P, and −0.09 ± 0.02 for placebo, p = 0.38 | Neither E alone nor E+P affected the progression of coronary atherosclerosis. |
| Waters et al. (55)/2002 (WAVE) | Randomized, double-blind, placebo-controlled secondary prevention trial | 423 postmenopausal women with at least 1 coronary stenosis 15% to 75%, mean age 65 yrs | CEE 0.625 mg ± MPA 2.5 mg daily (n = 210) vs. placebo (n = 213), and vitamins vs. placebo | 2.8 yrs | Quantitative coronary angiography: annualized mean change in minimum lumen diameter | Coronary progression worsened with HT by 0.047 (0.15) mm/yr and by 0.024 (0.015) in control subjects, p = 0.17. Death, nonfatal MI, or stroke HR was 1.9 (95% CI 0.97–3.6) in HT compared with control subjects. | No significant change in progression of atherosclerosis. Neither HT (nor antioxidant supplements) provided CV benefit. Instead a potential for harm was suggested. |
| Hodis et al. (56)/2003 (WELL-HART) | Randomized, double-blind, placebo-controlled | 226 postmenopausal women with CHD, mean age 63.5 yrs | Oral 17 β-estradiol (1 mg/day) alone (n = 76), +5 mg MPA (n = 74), or placebo (n = 76) | 3.3 yrs | Quantitative coronary angiography: average per-participant change in percent stenosis | Mean change in stenosis was 1.89 ± 0.78 in placebo, 2.18 ± 0.76 in E, 1.24 ± 0.80 in E+P; p = 0.66 for comparison | Estrogen alone or with progesterone had no significant effect on the progression of atherosclerosis. |
| de Kleijn et al. (53)/2001 | Randomized, double-blind, placebo-controlled primary prevention trial | 105 healthy postmenopausal women | Tibolone (n = 35), CEE+MPA (n = 35), or placebo (n = 35) | 3 months | Brachial reactivity: % flow-mediated lumen diameter change after 3 months | CEE+MPA vs. placebo had 2.5% change (0.3–4.6). Tibolone vs. placebo was 0.6% (−1.6–2.8). | HT with CEE+MPA (but not tibolone) increases endothelium-dependent flow-mediated dilation. |

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**Table 2.** Continued

| Investigator/ Year | Study Design | Subjects | Intervention | Median Follow-Up | End Point | Results | Conclusions |
|---|---|---|---|---|---|---|---|
| Angerer et al. (59)/2001 (PHOREA) | Randomized, double-blind, placebo-controlled, secondary prevention | 321 healthy postmenopausal women with increased carotid IMT | 17 β-estradiol 1 mg + 0.025 mg gestodone for 12 days/months vs. every 3 months (low-progestin) vs. no HT | 48 weeks | IMT: maximum carotid IMT thickness | HT did not slow carotid IMT progression | 1 yr of HT did not slow progression of subclinical atherosclerosis in postmenopausal women at increased risk. |
| Hodis et al. (52)/2001 (EPAT) | Randomized, double-blind, placebo-controlled, primary prevention trial | 222 healthy postmenopausal women without CHD | Unopposed 17 β-estradiol (1 mg) (n = 111) or placebo (n = 111) | 2 yrs | IMT: rate of change in carotid artery IMT every 6 mo | −0.0017 mm/yr in E arm vs. 0.0036 mm/yr, placebo-estradiol difference in progression was 0.0053 mm/yr (0.0001–0.0105 mm/yr, p = 0.046) | Progression of subclinical atherosclerosis was slower in healthy postmenopausal women taking unopposed E, compared with placebo. |
| Byingtoni et al. (58)/2002 (HERS B-Mode substudy) | Randomized, double-blind, placebo-controlled secondary prevention trial | 362 postmenopausal women with CHD (subset of HERS trial) | CEE 0.625 + MPA 2.5 mg (n = 177) or placebo (n = 185) | Mean 3.8 yrs | IMT: temporal change in mean of 8 maximum IMT measurements | IMT progressed 26 μm/yr (18–34) in CEE+MPA group and 31 μm/yr (21–40) in placebo group, p = 0.44 | IMT progressed in both groups without significant difference. |
| Schulman et al. (60)/2001 | Randomized, double-blind, placebo-controlled trial | 293 postmenopausal women presenting with unstable angina enrolled within 24 h of symptom onset | IV 1.25 mg bolus then oral CEE 1.25 mg + MPA 2.5 mg × 21 days vs. IV bolus then oral CEE 1.25 mg + placebo vs. IV then oral placebo | 48 h | ECG evidence of ischemia by continuous ambulatory monitoring (first 48 h) and repeated after 21 days of study drug | ECG ischemia did not differ among the three groups | Acute HT does not reduce ischemia in postmenopausal women with unstable angina when added to standard anti-ischemia therapy. |
| Viscoli et al. (61)/2001 (Women's Estrogen for Stroke Trial) | Randomized, double-blind, placebo-controlled secondary prevention trial | 664 postmenopausal women (mean age 71 yrs) with recent stroke or TIA | 17 β-estradiol 1 mg | Mean 2.8 yrs | CV events: recurrent stroke or death | Combined events: RR 1.1 (0.8–1.4) in E vs. placebo. Death alone RR 1.2 (0.8–1.8). Nonfatal stroke RR 1.0 (0.7–1.4). Fatal stroke RR 2.0, (0.9–9.0) in E users. Nonfatal strokes had slightly worse neurologic outcome in E users. | Estradiol does not reduce mortality or recurrent stroke in postmenopausal women with cerebrovascular disease. |

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**Table 2.** Continued

| Investigator/ Year | Study Design | Subjects | Intervention | Median Follow-Up | End Point | Results | Conclusions |
|---|---|---|---|---|---|---|---|
| Hulley et al. (5)/1998 (HERS) | Randomized, double-blind, placebo-controlled secondary prevention trial | 2,763 postmenopausal women with CHD, mean age 66.7 yrs | CEE 0.625 + MPA 2.5 mg (n = 1,380) or placebo (n = 1,383) | Mean 4.1 yrs | CV events: occurrence of nonfatal MI or CHD death | RR of 0.99 (0.80–1.22). Trend to increased events in first year with fewer events at years 4–5. | No overall CV benefit. Possible divergence at 4–5 yrs. HT increased the rate of thromboembolic disease. |
| Grady et al. (62)/2002 (HERS II) | As above | As above | As above | Mean 6.8 yrs | CV events: as above | Unadjusted RR of 0.99 (0.81–1.22), adjusted 0.99 (0.84–1.17) | Lower rates of CHD events at 4–5 yrs among women on HT in HERS did n ot persist during additional years of follow-up. |
| Clarke et al. (57)/2002 (PHASE) | Randomized, prospective secondary prevention trial | 255 postmenopausal women with ≥1 coronary stenosis >50%, mean age 66.5 yrs | Transdermal E+P (n = 134) vs. placebo (n = 121) | 30.8 mo | CV events: MI, cardiac death, or admission to hospital with unstable angina | Event ratio 1.29 (0.84–1.95, p = 0.24) | HT group had a not statistically significantly higher event rate compared with control subjects. |
| Roussouw et al. (6)/2002 (WHI CEE and MPA) | Randomized, double-blind, placebo-controlled primary prevention trial | 16,608 postmenopausal women | CEE 0.625 mg + MPA 2.5 mg (n = 8,506) vs. placebo (n = 8,102) | 5.2 yrs (planned 8.5 yrs) | CV events: primary CHD outcome of nonfatal MI, and CHD death; adverse risk score included invasive breast cancer | HR: CHD 1.29 (1.02–1.63), breast cancer 1.26 (1.0–1.59), stroke 1.41 (1.07–1.85), PE 2.13 (1.39–3.25), colon cancer 0.63 (0.43 –0.92), endometrial cancer 0.83 (0.47–1.47), hip fracture 0.66 (0.45–0.98), total mortality 0.98 (0.82–1.18) | Stopped early for absolute excess risks. For 10,000 person-years attributed to HRT were 7 more CHD events, 8 more PEs, 8 more asive breast cancers; whereas 6 fewer colon cancers and 5 fewer hip fractures were seen. |
| Anderson et al. (63)/2004 (WHI-CEE alone) | Randomized, double-blind, placebo-controlled trial primary prevention | 10,379 postmenopausal women with prior hysterectomy | CEE 0.625 alone (n = 5,310) vs. placebo (n = 5,429) | 6.8 yrs | CV events: as above | HR for CHD events 0.91 (0.75–1.12), breast cancer 0.77 (0.59–1.01), stroke 1.39 (1.10–1.77), PE 1.34 (0.87–2.06), colorectal cancer 1.08 (0.75–1.55), hip fracture 0.61 (0.41–0.91), total mortality 1.04 (0.88–1.22) | The use of CEE increases the risk of stroke, decreases the risk of hip fracture, and does not affect CHD incidence. |

Abbreviations as in Table 1.

randomized trials looking at atherosclerotic progression by coronary angiography showed no benefit (54–56). In women who already had coronary disease (57) or increased subclinical atherosclerosis on carotid IMT assessment (58,59), HT had no impact on disease progression. Also in a study of postmenopausal women presenting with unstable angina, acute HT started in the hospital setting had no effect on reducing further ischemic events evaluated by ambulatory electrocardiographic monitoring (60). Similarly, HT given to women with recent acute stroke did not reduce subsequent stroke or mortality (61).

The Heart and Estrogen/Progestin Replacement Study (HERS) was the first published secondary prevention trial in 2,763 women with known CHD followed up for the primary outcome of the cardiovascular events of nonfatal MI or CHD death. At a mean of 4.1 years, there was no significant difference in the HT arm versus the placebo arm (hazard ratio of 0.99, 95% confidence interval [CI] 0.80 to 1.22) (5). There seemed to be a trend toward benefit at longer durations of therapy, but this was not supported with the longer follow-up period of seven years (hazard ratio of 0.99, 95% CI 0.81 to 1.22) (62).

The largest randomized clinical trial is the Women's Health Initiative (WHI), which included 16,608 women with an intact uterus randomized to CEE and medroxyprogesterone acetate (MPA) or placebo with a 5.6-year mean follow-up (6). This was stopped early because of an increase in breast cancer among HT users with no cardiovascular benefit. An additional 10,739 women with hysterectomy were randomized to CEE alone or placebo, and that trial also showed no cardiovascular benefit (63). The findings for the combined CEE-MPA arm suggested that for 10,000 person-years there would be seven more CHD events, eight more strokes, eight more pulmonary embolisms, and eight more invasive breast cancers. For the CEE alone group, there would be an absolute excess of 12 strokes per 10,000 people despite a risk reduction of 6 fewer hip fractures. The WISDOM trial (64), based in the United Kingdom with a design similar to the WHI trial, was discontinued in 2002 after the results of the WHI trial were published.

Randomized placebo-controlled trials reduce many of the biases inherent in observational studies but have other limitations. The WHI trial is a very important study that has changed the national view of HT. Although primarily a primary prevention trial, a small number of patients did have established disease, including a history of MI (1.6%), history of angina (2.8%), history of coronary bypass surgery/percutaneous coronary intervention (1.1%), history of stroke (0.7%), and/or CHD risk factors of diabetes (4.4%), hypertension on therapy (35.7%), and hyperlipidemia on therapy (12.5%). The mean age of 63 years puts the majority of these women at least 10 years after menopause at the time of initiation of HT. There was also significant crossover between the two arms. Of consideration, only one drug regimen, CEE 0.625/MPA 2.5 mg orally per day, was tested, so these findings may or may not apply to lower dosages of these drugs, other formulations, or other routes of delivery.

## QUESTIONS TO BE ANSWERED

Given the findings of the HERS and of WHI trials, is the HT discussion ended for good? Hardly. The conflicting results from animal/observational studies compared with the randomized controlled trials raise many unanswered questions. These include whether some of the discrepancy is related to the age of the women in the studies, the timing of initiation (perimenopausal or postmenopausal), the amount of atherosclerosis at the time of initiation (primary vs. secondary prevention), the dosage, and the preparation form (transdermal, oral, or intravenous with or without progesterone), and whether there are genetic aspects to benefit or harm from HT.

Recently, Prentice et al. (65) reanalyzed the data from the observational trials adjusting for time from estrogen-plus-progestin initiation and confounding variables, and found that the readjusted hazard ratio estimates between the observational and experimental trials became much more similar for outcomes of CHD and thromboembolism, although less so for stroke (65). These analyses suggest that the apparent discrepancies between clinical trial and observational study findings may be substantially explained by classical confounding and differences in distributions of time of initiation.

**Timing of initiation.** Because atherosclerosis accelerates after estrogen deficiency, it would seem logical that estrogen replacement would have the most benefit when starting early in perimenopausal women. Most women in the observational trials such as the Nurses' Health Study, which suggested a protective effect of estrogen, started HT during the perimenopausal transition (66), whereas the WHI trial contained too few women in the perimenopausal period to evaluate whether any cardiac protection was seen. In the WHI trial the average age was 10 years after menopause, an age at which subclinical atherosclerosis has developed in many women (67).

In support of the notion that timing of initiation is critical, animal studies also showed no benefit of estrogen in animals that already had artery damage, either from balloon injury or from atherosclerotic diet, before initiation of HT (68). These animal studies are consistent with the findings of the secondary prevention (i.e., HERS) trials. In postmenopausal women in the Cardiovascular Health Study, estrogen replacement only caused vasodilation of the brachial artery in younger women without clinical or subclinical cardiovascular disease, suggesting that the favorable effects of estrogen may be limited to only those in whom atherosclerotic vascular disease has not yet developed (69).

The Kronos Early Estrogen Prevention Study (KEEPS) is a multicenter randomized placebo-controlled clinical trial that will evaluate the effectiveness of 0.45 mg CEE or 50 $\mu$g transdermal estradiol (in combination with 200 mg proges-

terone) in preventing the progression of carotid IMT or coronary calcium in women who are within 36 months of their final menstrual period (70). It is hoped that the KEEPS trial will provide some answers to the important question of whether HT will have a beneficial role if started early, although a relatively small sample size and the use of a surrogate end point represent limitations of this study. Hodis et al. (71) have recently launched the Early vs. Late Intervention Trial with Estrogen (ELITE), which is also focused on examining the potential importance of time since menopause on the cardiovascular effects of HT. In the ELITE study, the effects of oral 17$\beta$-estradiol on carotid IMT will be compared directly in perimenopausal women versus those >6 years after menopause.

**Dose.** Although 0.625 mg CEE clearly showed no cardiovascular benefit in the HERS and WHI trials, the observational Nurses' Health Study found the protective effect of CEE only in the lower doses of 0.3 mg and 0.625 mg, whereas 1.25 mg and higher doses were not protective. In a small randomized, double-blind crossover trial by Koh et al. (13) of 57 postmenopausal women on progesterone, lower-dose CEE 0.3 mg compared with 0.625 mg had similar favorable effects on HDL, triglycerides, and brachial reactivity, but had fewer prothrombotic effects and a smaller increase in CRP. In postmenopausal diabetic women without a recent MI among the Kaiser Permanente database, low-dose or medium-dose estrogen (<0.625 mg) decreased the risk of MI, which was not seen with a higher dose (72). Whether a lower dose of estrogen such as 0.3 mg CEE would provide cardioprotection without increasing thromboembolism remains to be seen.

**Route of delivery.** The formulation of estrogen used in the large clinical trials and in the majority of the smaller studies was CEE with or without MPA. There are extremely limited randomized trial data for other preparations of HT. Transdermal estrogen delivery provides sustained release of estrogens and more constant blood levels than oral administration. When estrogen is given orally, it has first-pass effects on the liver. Transdermal preparations avoid the first-pass effects on the liver and have less effect on the lipoprotein profile (73). Estradiol-to-estrone conversion is slower in parental administration, but transdermal delivery more commonly facilitates an estradiol-estrone ratio of about 1, which is similar to the physiological ratio in the pre-menopausal state.

Other differences that favor the transdermal approach include a neutral effect on CRP, a decrease in factor VII and fibrinogen, and a reduction in blood pressure (74). Because the first pass through the liver is avoided, there may be less induction of a prothrombotic state with the transdermal preparation. The Estrogen and Thromboembolism Risk study Group (ESTHER) case-controlled trial found that oral estrogen but not a transdermal formulation increased the risk of venothromboembolism in postmenopausal women on HT compared with control subjects (75).

In a case-controlled study, transdermal users seemed to have similar level of cardioprotective effects as those receiving oral preparations (46). These effects are not significant, but this may be attributable to small sample size. However, an animal study using transdermal estradiol did not find inhibition of aortic atherosclerosis (76).

Overall, the benefits of a transdermal estrogen preparation over an oral one seem encouraging, but further randomized trials are warranted (77). It is hoped that the KEEPS trial (70), which will randomize healthy perimenopausal women to oral versus transdermal hormone replacement, will provide information on this important question.

**Genetics.** It is possible that genetically determined subgroups of women may benefit by or be harmed from HT. Studies have shown that the cardiovascular effects of HT differ in individuals with specific genetic variants of certain genes such as apolipoprotein E4 (−) and myeloperoxidase (78,79). Recent work has also shown that genetic variation in the estrogen receptor itself may modulate the cardiovascular effects of HT, and also alter the underlying risk of CHD. The Herrington laboratory recently showed that a specific genetic variant of ER$\alpha$ is associated with an enhanced HDL-increasing effect of HT (80). Quite surprisingly, this same genetic variant of ER$\alpha$ was also recently shown to be associated with an approximately three-fold increased risk of MI in men in the Framingham heart study (81).

**Statin use.** Perhaps concomitant statin use may attenuate the negative cardiovascular effects of HT. Subgroup analysis of the HERS trial showed that the increased cardiovascular risks of HT in this population of women with established CHD did not occur in women taking statin therapy; however, there was no incremental risk reduction for cardiovascular events in women on both statin and HT compared with statin alone (82).

**Summary.** In conclusion, the HT controversy has not yet been laid to rest. Current randomized clinical trial data support the American Heart Association/American College of Cardiology guidelines that HT should not be prescribed for prevention of cardiovascular disease (83,84). However, it remains possible that some formulations and doses of HT may have favorable cardiovascular benefits when initiated earlier in the premenopausal or perimenopausal period in women without pre-existing atherosclerotic disease. We await the KEEPS and ELITE trial results, among others, to further answer this important issue.

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